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Preformulation studies of ceftriaxone for pediatric non-parenteral administration as an alternative to existing injectable formulations

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ABSTRACT

Ceftriaxone, a third generation cephalosporin, has a wide antibacterial spectrum that has good CNS penetration, which makes it potentially suitable for initial treatment of severe neonatal pediatric infections providing suitable formulation. We evaluated its physicochemical and technical characteristics to assess its potential for development as a non-parenteral dosage form. As ceftriaxone is marked only for injectable use, these data are not available. Using HPLC and Karl Fischer titration, sensitivity of ceftriaxone to water, feasibility and impact of pharmaceutical processes and compatibility with common pharmaceutical excipients were assessed. X-ray diffraction studies gave deeper insight into the mechanisms involved in degradation. Chemometrical analysis of near infrared spectra enabled classification of ceftriaxone powder according to exposure conditions or processes applied. The results showed that ceftriaxone was not highly hygroscopic, could be processed in all climatic zones, but should be packaged protected against humidity. Controlling water presence in formulation was shown critical, as ceftriaxone degraded in the presence of water content above 2.4% w/w. To improve flowability, a critical parameter for dry dosage form development, granulation (wet and dry techniques, providing complete drying and moderate force compaction respectively) was shown feasible. Compression with moderate forces was possible, but grinding and high compression forces significantly affected long term ceftriaxone stability and should be avoided. Based on these results, development of ceftriaxone non-parenteral solid or liquid non-aqueous forms appears feasible.

1. Introduction

Ceftriaxone (CTX) is a hemi-synthetic broad-spectrum third-generation cephalosporin antibiotic (Cho et al., 2004). According to the European Pharmacopoeia monograph (European Pharmacopoeia 9.0, 2017), it appears as a yellowish or almost white slightly hygroscopic crystalline odourless powder, freely soluble in water but sparingly soluble in methanol and very slightly soluble in anhydrous ethanol. The ceftriaxone sodium pharmaceutical raw material is the disodium salt hemiheptahydrate of ceftriaxone. Thus, the molecular weight of CTX is 661.6 g/mol in its hydrated form, compared to 598.6 g/mol in its anhydrous form.

CTX is reportedly hygroscopic, sensitive to humidity, heat and light; oxidizing agents and heavy metals should be avoided (Roche, 2006). CTX aqueous solution is reportedly unstable; stability is pH and temperature dependent and degradation occurs according to multiple pathways (de Diego Glaria et al., 2003; de Diego Glaria and Moscciati, 2010; Gaudin et al., 2015; Sar et al., 2006; Zajac and Muszalska, 1998). In solution, the optimal pH for CTX stability is 7.5 giving > 6 h without significant degradation at 37 °C, however degradation is faster at lower or higher pH (Gaudin et al., 2015). A 10% aqueous solution CTX has a pH between 6.0 and 7.5 (Roche, 2006). CTX possesses two acidic groups and one basic group of which pKa were reported (Jenke, 1994, de Diego Glaria et al., 2005): pKa1 = 2.6 to 3 (carboxylic group);

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pKa2 = 3.2 (aminothiazole); pKa3 = 4.1 to 4.3 (enolic group). Thus, at physiological pH, CTX possesses two negative charges and is highly hydrophilic (Zhang et al., 2005a). It has a low octanol/water partition coefficient (log $P = -2.1 \pm 0.2$) (Lee et al., 2005). Consequently, it is poorly absorbed through mucosal membranes, which explains why the only pharmaceutical form is injectable. Its high aqueous solubility and low permeability classify this antibiotic in class 3 of BCS (Biopharmaceutics Classification System). It is reportedly sensitive to degradation in gastric fluid and is poorly absorbed from the intestinal tract (Cho et al., 2004).

Its antibacterial spectrum covers both Gram positive and Gram negative bacteria, which makes it suitable for antibiotic treatment of severe pediatric infections such as neonatal sepsis. Neonatal sepsis is an invasive bacterial infection occurring in the first 90 days of life. Its incidence is highest in situations related to poverty including maternal infections, low-birth-weight, obstetric complications (early onset, caused predominantly by group B streptococci and Gram negative enteric bacilli (*Escherichia coli*)) or infections acquired from the environment (late onset). Mortality can be as high as 40% (World Health Organization, 2003; Wiese, 1988; Fujii et al., 1988).

CTX efficacy in critically ill patients is dependent upon the time during which free drug concentrations in serum remain above minimum inhibitory concentrations (MIC) of the invading microorganism; ideally trough concentrations should be constantly above the MIC of the pathogen, and for > 50% of the dosing interval (Downes et al., 2014). Because of age-dependent drug-binding proteins, CTX elimination half-life is age-dependent: 15 to 19 h in 1–8 day neonates and 4 to 6 h at 12 months. The volume of distribution in neonates is ~0.45 L/kg. CTX excretion is by the urinary and biliary routes (40–60% and 10–20% respectively) (Theriaque, 2016).

Currently, CTX exists on the market as disodium hemiheptahydrate salt. This was initially commercialized by Hoffman la Roche Inc. under the name of Rocephine®, but is now out of patent and available in several generic pharmaceutical products. The only current pharmaceutical drug dosage form is CTX powder for extemporaneous reconstitution of injectable solution for intravenous (IV), intramuscular (IM) or sub-cutaneous (SC) administration (Theriaque, 2016), which limits its therapeutic use to medical facilities. IM and SC administrations are bioequivalent to IV administration (Hayward et al., 1996; Harb et al., 2010).

Several formulations have been developed to improve CTX absorption. Most are based either on CTX complex formation (Lee et al., 2005, 2006; Cho et al., 2004) or on CTX encapsulation in the form of mucin-gelatin mucoadhesive microspheres (Ofokansi et al., 2007), lipospheres (Attama et al., 2009) or alginate encapsulated particles (Debrouse, 2009, patent US20090123537 A1; Patel et al., 2016). Improvement of CTX bioavailability from each of these formulations (except for lipospheres and enteric coated alginate beads -evaluated only in vitro) has been shown in rodents or rabbits after oral or rectal administration.

In view of the importance of simplifying antibiotic treatment of critically ill newborns before they reach health facilities, this article focuses on CTX preformulation studies of solid and liquid non-parenteral drug dosage forms for further pharmaceutical development.

2. Material and methods

2.1. Material

Ceftriaxone was provided by Roche (batch no. 100193830405973001) or Discovery Fine Chemicals (batch no. 73777) and complied with the European Pharmacopoeia 9th ed. monograph (European Pharmacopoeia 9.0, 2017). All excipients were of pharmaceutical grade, purchased from common pharmaceutical suppliers. All chemicals and solvents used were of analytical grade, purchased from Sigma-Aldrich (France) or VWR (France).

2.2. Methods

2.2.1. CTX sample preparation

CTX was studied under various conditions, as raw material (flowability, solubility, control condition for exposures) or exposed to different atmospheric conditions simulating various climatic zones, different quantities of water simulating formulations containing some water, or different pharmaceutical operations. After CTX exposure, samples were blistered in alu/alu blisters and underwent accelerated stability testing (40 °C/75% RH) with analytical controls at defined times (visual inspection, HPLC defined CTX content, Karl Fischer defined water content, NIR, PXRD) and compatibility testing with excipients, as described below.

2.2.1.1. Exposure of CTX to various humidity/temperature conditions. CTX powder was exposed to different conditions simulating ambient humidity in various climatic zones. Samples of 4 g of CTX powder were accurately weighed in an aluminium weighing pan. The samples were stored in a climatic chamber (Firlabo SP540_BVEHF) according to following ICH conditions for temperature and humidity:

- 25 °C/60% RH for 6 h or 24 h of exposure time
- 25 °C/75% RH for 6 h or 24 h of exposure time
- 40 °C/75% RH for 6 h or 24 h or 7 days of exposure time

The duration of exposure of 24 h or 7 days was chosen to mimic likely climatic exposure during pharmaceutical operations. The samples underwent three-dimensional agitation to ensure their homogeneity using Turbula apparatus at the end of the exposure period and then blister packed in aluminium blisters for immediate analysis or further stability studies.

2.2.1.2. Wetting of CTX. Water was sprayed on CTX samples (4 g) placed on a glass tray, using manual non-pressurized sprayer with plastic nozzle. Samples were accurately weighted before and after spraying to study the impact of water added during formulation process on CTX stability. Conditions with 0%, 5%, 10%, 15% and 20% weight/weight (w/w) of water added were studied. Wet CTX samples were mixed using Turbula apparatus at the end of spraying and blistered in aluminium blisters for immediate or for further stability analysis.

2.2.1.3. Simulation of pharmaceutical processes on CTX. The impact of several common pharmaceutical processes was studied. Each process was simulated as follows:

- *Grinding*: CTX powder was ground with a mortar and pestle for 5 min.
- *Compaction/crushing* (*simulation of dry granulation*): the compression chamber of tablet press (K0000114, Korsch Pressen, Germany) was filled manually. CTX powder contained in the compression chamber was then compressed. The tablets produced were then crushed with a mortar and pestle.
- *Compression*: Pure CTX tablets were made by direct compression using an instrumented hydraulic press (Perrier Labotest®, France). The compression chamber was manually filled with 200 mg CTX and compressed applying a pressure of 80 MPa or 150 MPa on the powder using flat faced round 10 mm punches.
- *Wetting/drying* (*simulation of wet granulation*): CTX powder was weighed, transferred in a mortar, sprayed with purified water (15% w/w or 30% w/w) and mixed with a spatula. The wet blend was dried in an oven at 40 °C over 24 h.

After preparation, all the processed simulation samples (grinding, compaction/crushing, compression and wetting/drying) were weighed, placed in a glass flask and homogenized in the Turbula® for 10 min at

67 rpm. Each sample was divided into 4 glass flasks to study CTX content at time 0 (T0), 1 month (T1 m), 3 months (T3 m) and 6 months (T6 m). Each glass flask was packaged and sealed in aluminium blisters.

2.2.1.4. Solubility studies of CTX in liquid excipients. For solubility studies of CTX in liquid excipients, 500 mg CTX was accurately weighed and progressively added to a 10 mL of liquid excipient under magnetic stirring, in closed glass vials. Each time that dissolution was achieved (crystal clear solution), a further amount was added.

2.2.1.5. Flowability of CTX powder. Flowability of CTX raw powder was tested according to European Pharmacopoeia (9th ed.). Briefly, 105 g of CTX powder was placed in the funnel of the flowability apparatus (Pharmatest, PTG-S4, Germany) with 25 mm, 15 mm and 10 mm nozzles. The apparatus registered the time necessary for 100 g of powder to flow through spontaneously when opening the nozzle onto the balance beneath. Three measures were taken. The time was considered infinite when 100 g failed to flow through.

CTX blended with dicalcium phosphate (Encompress, 8% w/w), colloidal silica (Aerosil, 0.5% w/w), talc (0.5% w/w) and stearate Mg (0.5% w/w) or a combination of all cited excipients were tested within the same protocol.

2.2.2. Stability studies of CTX samples

Samples were placed in a climatic chamber (Prolabo, France) at 40 °C/75% relative humidity (RH) which corresponds with ICH defined conditions for tropical zones (zone IV) for accelerated International Conference on Harmonisation (ICH) stability assessment. T0 samples were kept at 4 °C until analysis. At defined times (T0, T1m, T3m, T6m) samples were removed and stored at 4 °C until analysed.

2.2.3. HPLC

An ion pair reverse phase HPLC method was developed for CTX analysis. Briefly, the chromatographic separation was carried out on a reverse phase C18 column (YMC ODS-H80 150 × 4.6 mm, 4 µm) at 40 °C, and coupled to a diode array detector set between 220 and 330 nm. The flow rate was set at 1 mL/min and samples were analysed during 15 min after 2 h equilibration. The mobile phase was a combination of a tetramethylammonium bromide solution (18 mM, ion pairing agent) solubilized in a phosphate buffer (25 mM, pH 7.5) and mixed with methanol (72:28, % volume/volume (v/v)) under isocratic elution mode. For sample preparation, a preliminary dissolution step using methanol and/or phosphate buffer as solvents was applied, followed by a filtration step through membrane filter 0.45 µm. The method was validated according to the ICH guidelines. Assays were linear in the concentration range 24–36 µg/mL.

The method specificity was evaluated by injecting each excipient alone at the same concentration to that of CTX-excipient mixture. It was observed that none of the peaks appeared at the same retention time as the CTX peak. It was concluded that the HPLC method was selective in relation to the excipients of mixture preparations.

2.2.4. Karl Fischer

Water content in samples was measured by Karl Fischer volumetric titration method using a Titralab TIM 580 t system supplied by Radiometer analytical (Titration Manager). The system was equipped with a titration cell (100 mL), a 10 mL autoburette and a M 241 Platinum electrode. The Karl Fischer titrator was filled with Hydranal-Composite 5 reagent and was calibrated with 10 µL of purified water in order to determine the exact titre of the reagent (10.0 mg H_2O/g). The following Karl Fischer titrator parameters have been used: an electrode polarization of 50 mA, an end-point voltage of 200 mV, a maximum titration rate of 5 mL/min, a drift as stop criterion of 50 µg/min and at 500 t/min of magnetic stirring.

The Karl Fischer method for the determination of water content was used as follows: 40 mL of methanol was placed in the titration cell and titrated with Hydranal composite 5 as far as the end-point indicated by polarized electrodes. An accurately weighed amount of sample powder (from 100 mg to 150 mg) was then added into Karl Fischer cell, dissolved in the methanol flask for 10 min before titration. Then titration reagent was added to a new end point. The amount of water in analysed samples was displayed automatically and moisture content calculated. Triplicate titrations were performed for each sample.

2.2.5. Near infrared (NIR) spectroscopy

An FT-NIR analyser Antaris II (ThermoScientific Instrument, Madison, USA) was used to acquire all spectra. FT-NIR spectrometer was composed of a Michelson interferometer, a quartz halogen light source (50 W), a matched InGaAs detector and an Antaris II integrating sphere for diffuse reflectance. Spectra acquisition and treatment were performed using Result, TQ Analyst (version 6.21) and Omnic software (version 6.1a). Samples were analysed using a 1.5 mL glass vial (Chromacol, 10 mm in diameter, ThermoScientific Instrument). Powders were introduced in the glass vial placed on the sapphire window and scanned to acquire diffuse reflectance spectra. Acquisition parameters were an average of 32 co-added scans, 8 cm^{-1} of resolution, spectral range of 9999–4000 cm^{-1}, daily background spectra of room environment. For each sample, spectra were recorded in triplicate. Vials were turned in a three-dimensional motion manually to homogenise the sample before each acquisition.

2.2.6. Principal component analyses (PCA)

All PCA models were made using TQ Analyst software. Three models were built to evaluate the impact of exposure, water addition and pharmaceutical processes on CTX. All spectra were pre-treated using baseline correction and Standard Normal Variate (SNV). For the exposure model, the entire spectral range without further pre-treatment was required. Pre-treatment was not used to conserve the information on the physical properties of the samples. In the case of water addition and pharmaceutical process models, a restricted spectral range (7482.45 to 4666.89 cm^{-1}) was used to focus on specific zones of CTX and water. To enhance spectral information and enable a better separation, a Savitzky-Golay first derivative was applied on the chosen spectral range in the case of water addition model. The specific spectral region for water addition model was then selected between 7482.45 and 4666.89 cm^{-1}. In this zone, specific water absorption bands and functional groups of CTX are combined allowing a better discrimination.

2.2.7. X-ray diffraction

High resolution powder X-ray diffraction (PXRD) analyses were performed on a PANalytical X'Pert Pro diffractometer using a Cu anticathode and a primary monochromator (Ge 111) crystal to obtain a monochromatic radiation (λCu = 1.5406 Å). Routine diffractograms of CTX control powder and CTX preformulations were recorded using a PANalytical X'Pert Pro diffractometer composed of a graphite monochromator with CuKα radiation (λ = 1.5418 Å). The data were collected using reflection geometry with a 2-axis goniometer (theta/theta or theta/2theta) and a rotating sample holder to limit preferred orientation effects. The program DICVOL06 was used to index the X-ray pattern of CTX (Boultif and Louër, 2004). The diffractograms were analysed and simulated using the Le Bail algorithm (Le Bail, 2005) to extract the diffracted intensities and refined using the profile matching method with the FullProf Suite software (Rodriguez-Carvajal, 1993) or the program JANA2006 (Petricek et al., 2014). The microstructural parameters (crystallite size and strain) were extracted from PXRD diagrams by subtracting the instrumental peak broadening contribution according to the calibration with the LaB_6 NIST standard or by fundamental parameters approach.

2.2.8. Compatibility studies of CTX with excipients

A list of 87 potential excipients for further pharmaceutical devel-

opment was established. Excipients for solid, semi-solid and liquid formulations were preferentially (but not exclusively) considered from commonly used excipients in commercialized pediatric formulations (Table S1). Compatibility studies of binary mixtures CTX — excipients were performed as follows: accurately weighed amounts of CTX (500 mg, 600 mg or 750 mg) were mixed with each tested excipient either in a ratio 1:4 or in a 1:1 ratio depending on the excipient properties (Table S1). Each blend was divided into three fractions and stored in closed glass vials at common stability study conditions, i.e. at 4 °C for T0 condition, at room temperature (RT) and in an oven at 40 °C (T40) until 6 months. Fine powder excipients were mixed with the CTX directly in a Turbula® mixer at 67 rpm for 10 min. Flake and pellet excipients were manually ground with a mortar and pestle (5 min) prior to being mixed with the CTX in a Turbula® mixer at 67 rpm for 10 min. Liquid and oily excipients were mixed with the CTX by magnetic stirring until complete homogenization (10 min) was achieved, when each fraction was withdrawn whilst stirring. Semi-solid excipients and waxes were liquefied by heating 10 °C above the melting point and treated like liquid and oils. In some particular cases, for very viscous liquids, samples were co-ground with CTX until homogenisation (10 min). All samples were sub-divided and aliquots packed in aluminium blisters to facilitate stability studies.

The content determination of CTX in binary blends was measured by HPLC method after sample preparation as follows: an appropriate mass of the CTX blend with excipient equivalent to 30 mg of CTX was accurately weighed and dissolved in a 100.0 mL volumetric flask with phosphate buffer. A minimal sufficient fraction of methanol was added when necessary according to the solubility of excipient. The solution was filtered through nylon membrane 0.45 µm and was diluted to one tenth with phosphate buffer before injection into the HPLC system. The CTX content was analysed in triplicate and the presence of degradation product peaks evaluated. Excipients were considered compatible when CTX content was at 100 ± 10% after 6 months at 40 °C/75% RH and no degradation products observed compared to control CTX. They were considered as incompatible when CTX content was out of 100 ± 20% limit or, out of 100 ± 10% in presence of degradation products. If the content was outside the ± 10% CTX limit but within the 100% ± 20% limits and no degradation products observed, excipients were considered as second line excipients, to be further tested if used.

2.2.9. Statistical analysis

Results were statistically compared using Student *t*-test with $p < 0.05$ as a significance limit on Excel software.

3. Results and discussion

3.1. CTX raw substance characterization

Preliminary preformulation tests, namely flowing properties of the powder, appropriate water dosage method, water content of raw material, solubility in non-aqueous solvents and crystalline structure were performed on CTX raw material. CTX used for this preformulation study was a batch of ceftriaxone sodium raw material complying with the European Pharmacopoeia 9.0 monograph. It was a white odourless crystalline powder.

Our flowability study performed according to European Pharmacopoeia (9th ed.) showed that CTX did not flow freely from a funnel. The flowability was classified as taking an infinite time, as the entire sample (100 g) of raw CTX failed to flow through the nozzle. The addition of a diluent (Encompress), or common glidants/lubricants (colloidal silica, talc, stearate Mg) did not allow a complete flow through of the powder blend. The irregular morphological aspect of the powder (data not shown) might explain the poor flowability, which was therefore identified as a critical challenge for further dry dosage development.

Since CTX aqueous solutions were reported to be unstable (de Diego Glaria et al., 2003; de Diego Glaria and Moscciati, 2010; Gaudin et al. 2015), water content of formulations was identified as potentially critical to stability. CTX is a hydrate that contains 3.5 water molecules for one CTX molecule, which represents 9.5% w/w of water of its total weight. Accurate CTX water content could only be determined using the Karl Fischer method. Loss on drying could not be used instead of Karl Fischer as it could not detect bound crystalline water.

CTX is reportedly unstable in water. Its exposure to accelerated aging conditions (40 °C/75% RH) on a tray resulted in visual degradation and browning of the CTX powder after 2 months. Plastic blisters turned out to be insufficiently protective and degradation of CTX was observed (data not shown). Aluminium blisters were necessary to protect CTX from degradation for 6 months at 40 °C/75% RH while preserving its water content (98.1% ± 3% and 104.8 ± 5% for CTX and water content respectively after 6 months compared to T0 conditions).

CTX solubility was studied in various pharmaceutical solvents (cf. excipients cited for compatibility studies in supplementary data Table S1). None achieved solubility of 500 mg CTX dose in < 10 mL, which would be compatible with oral or rectal administration. This is consistent with the European Pharmacopoeia 9.0 CTX monograph where solubility was described as high in water but low in non-aqueous solvents.

In order to characterise the crystalline phase and the microstructure of CTX and follow its evolution under different conditions, a high resolution PXRD was performed on CTX raw material. The diagram was indexed and the unit cell parameters found were refined by profile matching methods. The refinement results that led to the best background corrected reliability coefficient (Rwp) gave the following monoclinic unit cell parameters: a = 15.439(6) Å, b = 18.534(5) Å, c = 4.765(1) Å, and β = 98.66(3)° leading to a cell volume of ca. 1348(1) $Å^3$. Even if the data quality was not sufficient to definitely validate this unit cell, it is worth noting that according to a reasonable density, 2 molecules should stand in the unit cell, which is compatible with a non centro-symmetric monoclinic space group as expected for a chiral compound. Additionally, this unit cell allowed reproduction of the PXRD diagram.

The microstructural parameters could be obtained from the peak broadening after subtracting the instrumental contribution. The crystallite mean size of CTX was estimated at ca. 70 nm and the micro-strains in the crystallites were estimated to ca. $25 \cdot 10^{-4}$. These values were further used as a reference for comparison with the CTX under various conditions.

3.2. Sensitivity of CTX to water

3.2.1. Sensitivity of CTX to water under atmospheric conditions (hygroscopicity)

The objective of this study was to evaluate the long-term stability consequences of exposure to water during processing. As CTX aqueous solutions were previously found to be unstable (de Diego Glaria et al., 2003; de Diego Glaria and Moscciati, 2010; Gaudin et al., 2015; Sar et al., 2006; Zajac and Muszalska, 1998), CTX water content and CTX stability were monitored when exposed to various environmental conditions (temperature and relative humidity). The impact on CTX content, moisture content and stability (simulated by accelerated aging study of exposed and blistered CTX) was observed.

At T0, for all tested conditions, a longer exposure of CTX to ambient humidity (6 h, 24 h or 7 days, results not shown for 6 h) did not result in higher water uptake (Fig. 1). At 25 °C/60% RH compared to the control, no major water uptake was observed (< 0.2%). In contrast, when CTX was exposed to the same temperature but higher humidity there was a small but significant uptake of water (3.1% and 1.8% compared to control for 25 °C/75% RH and 40 °C/75% RH conditions respectively, *p*-value < 0.05). Thus, CTX did not appear to be a hygroscopic drug. Surprisingly, the initial water uptake just after

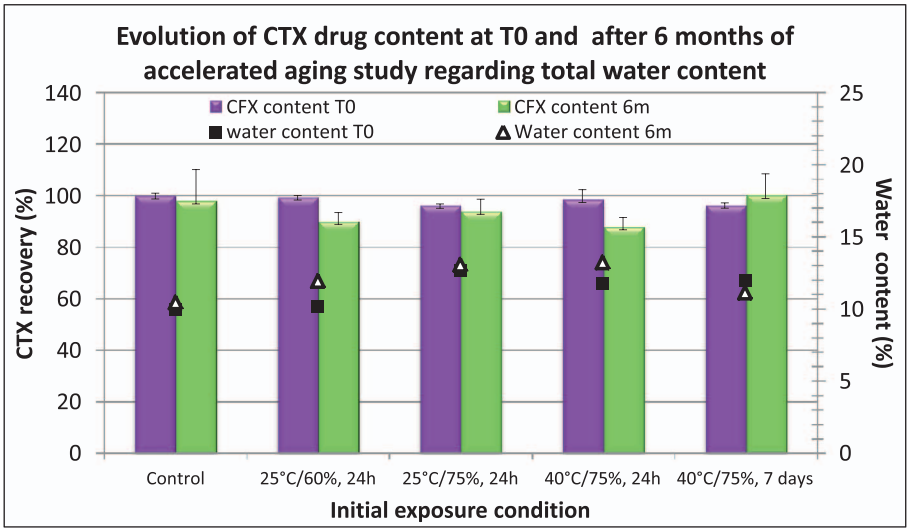

Fig. 1. CTX and water content at T0 and after 6 months of accelerated aging study for various atmospheric conditions of CTX powder initial exposure.

exposure was higher at 25 °C/75% RH compared to 40 °C/75% RH. This could be related to the thermal properties of CTX described in the literature (Zhang et al., 2005b). As the first dehydration step starts around 40 °C, it is possible that CTX starts to lose water at 40 °C, leading to a lower water uptake compared to 25 °C.

All (HPLC determined) CTX content results were considered similar regardless of the initial exposure or accelerated aging. NIR spectra of humidity and temperature exposed CTX samples were further analysed. The 3D scatter plot highlighted the fact that during an exposure to 25 °C/75% RH, water uptake was higher than in the 25 °C/60% RH condition (Fig. 2). These results corroborated Karl-Fisher titrations: initial exposure had an influence on CTX water uptake. Furthermore, samples submitted to accelerated aging were characterised by higher PC3 scores at 6 months compared to T0 condition. Since no major variations in water content were observed between T0 and 6 months for each specific condition (CTX or 25 °C/60% RH or 25 °C/75% RH), this increase of PC3 score could be related to some physical changes.

Exposure to humidity did not alter the CTX long term stability as demonstrated by HPLC although it led to significant (small) water uptake, and some physical changes observed in NIR. These results confirm that local production could be considered in countries in various climatic zones.

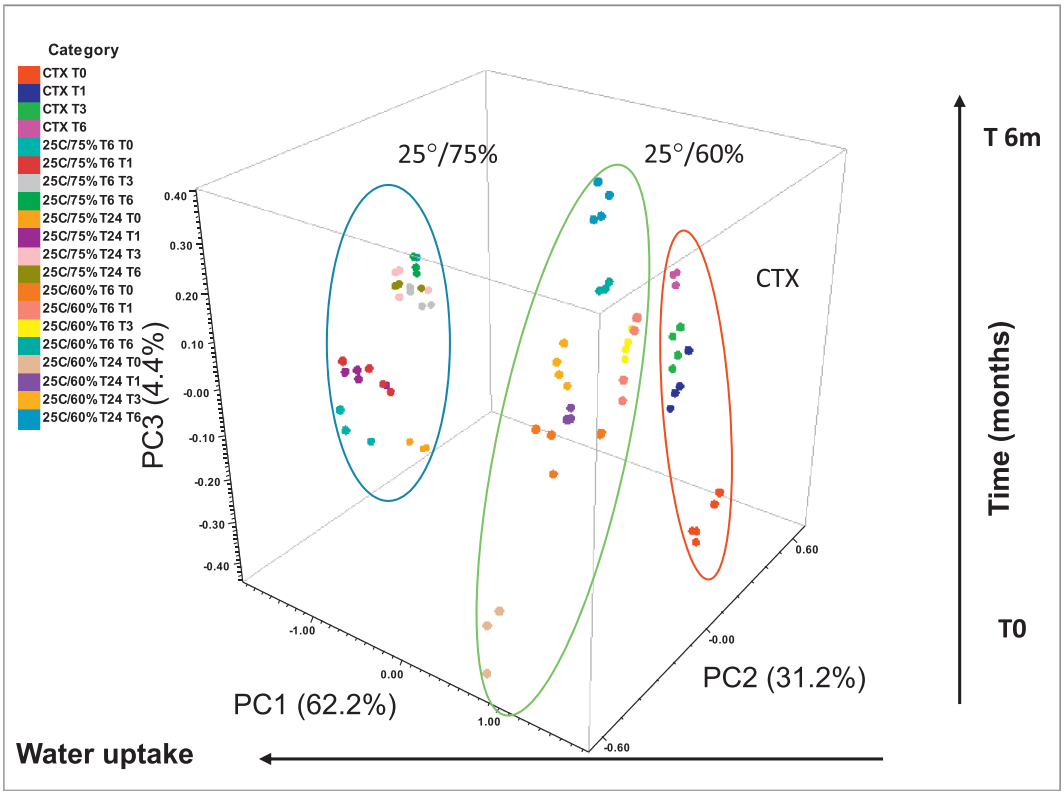

Fig. 2. Scatter plot of PCA model representing the impact of exposure conditions on CTX. Percentage of variance explained by first, second and third principal components is given in parenthesis (62.2% for PC1, 31.2% for PC2 and 4.4% for PC3). Spectra were pre-treated using SNV and 1st derivative before using PCA.

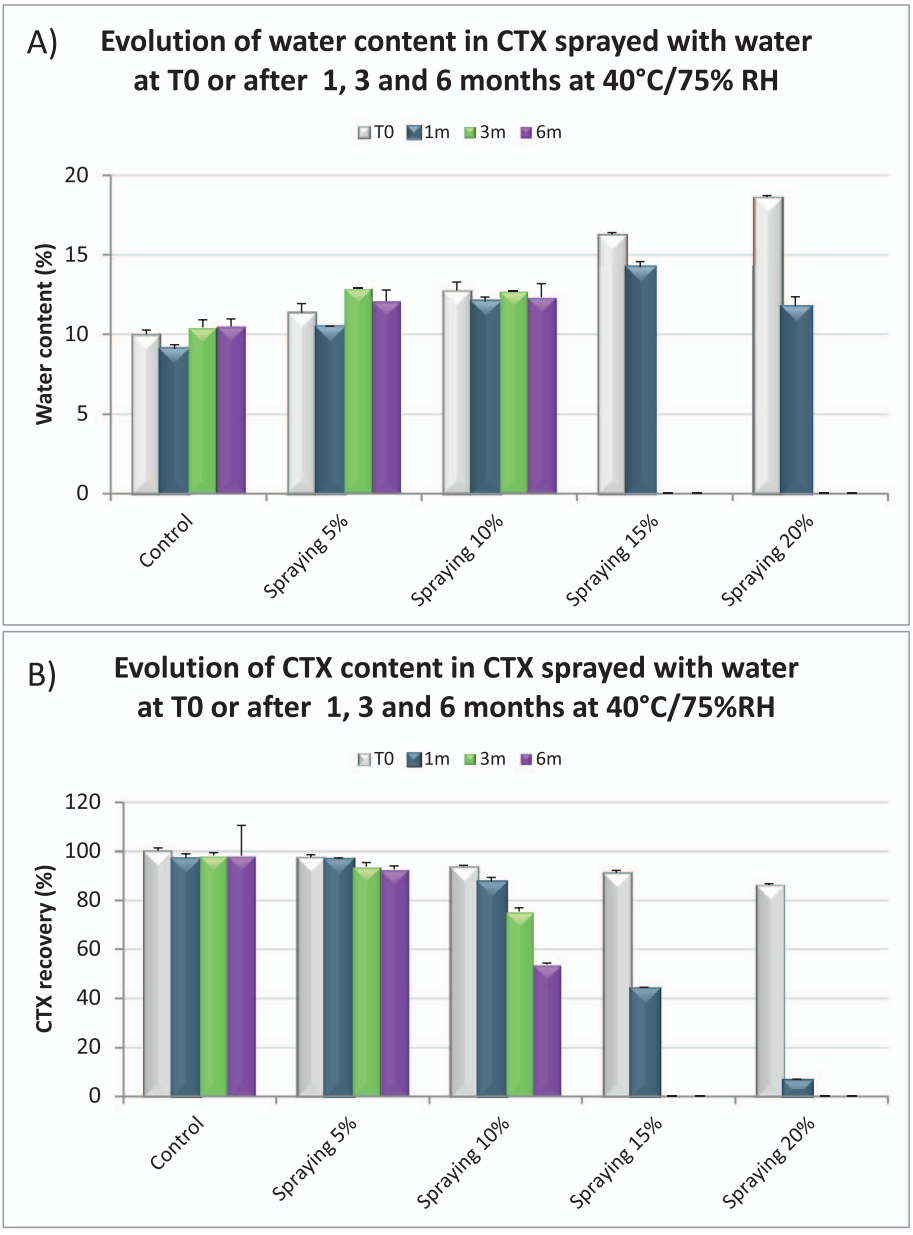

Fig. 3. Impact of water spraying over CTX powder on A) recovered water content and B) CTX loss during accelerated aging (40 °C/75% RH). For each of sprayed water amount, each column represented CTX recovery at T0, T 1 month, T 3 months and T 6 months respectively. *p-Value < 0.01 compared to the control; **p-value < 0.05 compared to the control.

3.2.2. Sensitivity of CTX to water added

When different quantities of water (5 to 20% w/w) were sprayed over CTX powder, immediate and long term stability of CTX was measured from T0 to 6 months in an accelerated stability study and the exact water content was analysed using the Karl Fischer method for all conditions at all time points (Fig. 3). Some samples (15% water and 20% water sprayed) at 6 months were not analysed or included in the stability and NIR study because of massive visual degradation.

The results showed that the more water was sprayed, the more water was recovered in CTX samples, at T0 and at every stability time point. Higher percentages of water dried partially during stability, but remained above control conditions. The more water was added, the more CTX degradation occurred. All conditions and all times showed degradation with significant decreases of CTX content ($p < 0.05$) except the condition where 5% water was sprayed ($p > 0.05$ for all times T0 to 6 months).

NIR spectra of CTX control and CTX with water addition during their accelerated aging were used for PCA. As presented in Fig. 4 water influence was shown with the distinction of 5 groups: CTX, CTX with 5%, 10%, 15% and 20% water addition. All CTX control samples were similar, showing good stability during the accelerating aging (T0, 1 month and 3 months) as shown by the HPLC results. However, the results from the four other conditions at T0, 1 month and 3 months were well separated indicating an alteration (physical and/or chemical) with PC1 decreasing and PC2 increasing considering one specific group. This model permitted rapid characterization of water content and degradation of CTX samples and is likely to be useful during future formulation assessments.

In summary, water added by spraying could be adequately monitored using Karl Fischer and NIR techniques. CTX long term stability was not significantly affected by the initial addition of 1.4% w/w of water (corresponding to 5% sprayed water, partially dried). However, CTX degradation was significant from T0 after 2.8% of water was added (corresponding to 10% sprayed water). CTX sensitivity to water was confirmed as high, excluding any possibility of formulating CTX in the presence of water. Water adsorbed by any excipients (e.g. cellulose

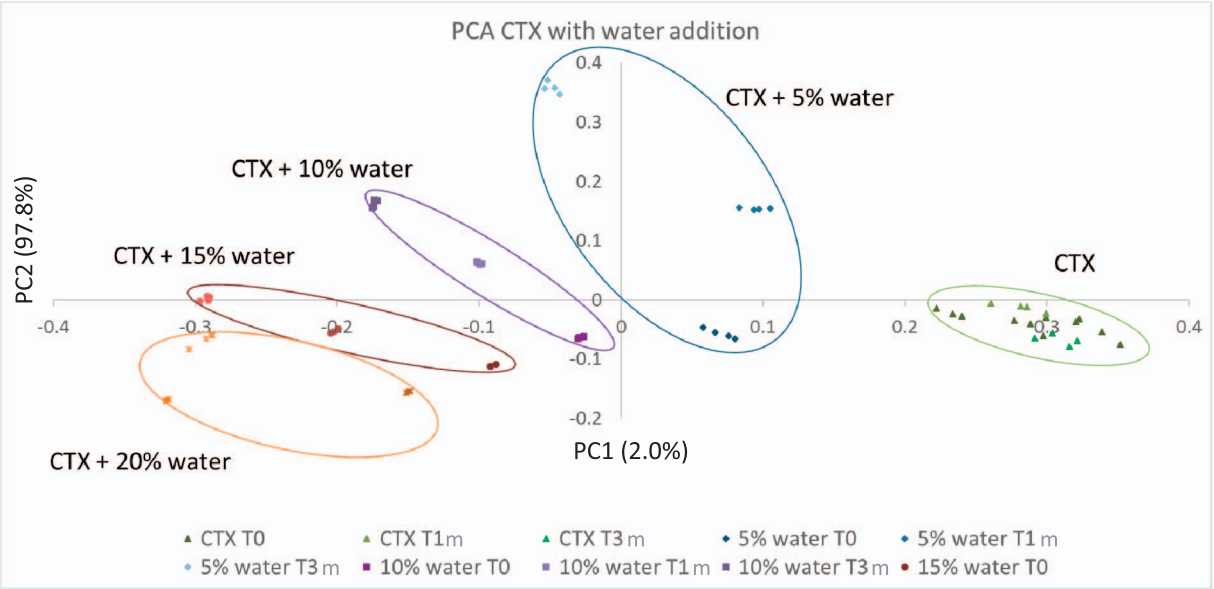

Fig. 4. Scatter plot of PCA model for water addition influence on CTX. Percentage of variance explained is given in parenthesis for first and second principal components (97.8% and 2.0% for PC1 and PC2 respectively).

derivatives) or contained in some hydrophilic solvents (ethanol, PEG, etc.) could potentially cause CTX degradation. Therefore, further studies on binary mixtures were conducted.

3.3. Sensitivity of CTX to common pharmaceutical operations

Several pharmaceutical processes were simulated to evaluate their effects on CTX immediate and long term stability. Grinding was studied to see the impact of physical forces on CTX integrity. Compaction – crushing was considered in the simulation of dry granulation process – in this case two processes were present, the compaction and crushing, which was performed in a mortar and considered similar to milling. Wetting and drying was tested to simulate the wet granulation process, using two different quantities of water, 15% or 30% *w*/w. Water content, NIR spectra and HPLC content were monitored immediately (T0) and during 6 months of the accelerated stability study.

Water content of processed CTX did not vary significantly compared to control (non-processed) CTX for all tested processes. As pharmaceutical processes impacted physical properties of CTX samples, NIR appeared to be a suitable analytical technique. Pre-processing was reduced to only SNV and baseline correction to preserve information related to physical properties. The PCA model highlighted the impact of pharmaceutical processes (Fig. 5). Firstly, the model was divided in two parts based on the PC1 score: one containing CTX control and grinding, and the other with conditions simulating granulation (either dry or wet). Secondly, CTX control and grinding samples were separated by PC2 scores. The PCA model enabled us to separate samples based on pharmaceutical process applied. This model was based on physical characteristics of CTX and for this reason does not reflect specifically CTX degradation over time.

HPLC determined CTX content was monitored to check CTX chemical stability and degradation. Results showed (Fig. 6) that CTX content did not differ significantly at T0 for any of processes tested. However, after 6 months of an accelerated aging stability study, CTX content of grinding and compaction/crushing conditions were significantly affected ($p < 0.05$), resulting in a moderate, but significant loss of CTX assay of 23% and 20% respectively. Consequently, before considering a size reduction of CTX raw material, further studies would be necessary to establish non-degrading conditions.

The loss in compaction/crushing condition corresponded with the results from grinding alone, and supported the hypothesis that grinding was the main cause. To check this point, compacted CTX was analysed for its content up to 6 months in an accelerated stability study. The results showed an absence of CTX degradation with 100 ± 1.6% CTX content after 6 months at 40 °C/75% RH compared to control (non-compressed CTX). Consequently, it was hypothesised that the CTX loss observed was related to grinding used to crush compacted CTX. Dry granulation remained a possible technique, but attention needs to be paid to the forces applied to crush CTX. Further studies would be necessary to characterise the optimal process parameters, if this technique was to be considered in formulating CTX dosage forms.

When samples were dried well (water content not higher than initially present in CTX raw material), wetting did not affect CTX long term stability. Therefore, wet granulation remains a viable alternative to solve CTX flowability problems.

Taken together, these data show that pharmaceutical processes tested did not affect CTX water content, but induced some physical and/or chemical changes, as shown by NIR and HPLC results. Wet or moderate compaction dry granulation processes could be considered to improve CTX flowing properties but grinding appeared risky for CTX long term stability.

3.4. PXRD analysis of processed CTX samples

In order to investigate changes in the crystalline phase and the microstructural parameters of CTX under common pharmaceutical operations, several PXRD diagrams were recorded. Routine PXRD diagrams are shown in Fig. 7a for CTX after grinding, after wetting with 20% of water, after a compression of 80 MPa and 150 MPa, with and without preliminary crushing and compared to CTX control as the reference. Additionally, high resolution PXRD were recorded after wetting with 20% of water, wetting with 30% of water and drying, and after compression in a tablet to extract the microstructural parameters.

As shown in Fig. 7a, all the peaks observed in the CTX control PXRD diagram remained at the same position under different conditions. Consequently, there was no structural transition or modification of the unit cell parameter observed immediately after the different tested conditions. Nevertheless, all the diagrams presented slight differences. Some variations of peak intensities, and some broadening of peaks were clearly observed in diagrams b to g of Fig. 7a. The difference in the peak intensity, mostly observed for CTX under compression (Fig. 7a diffrac-

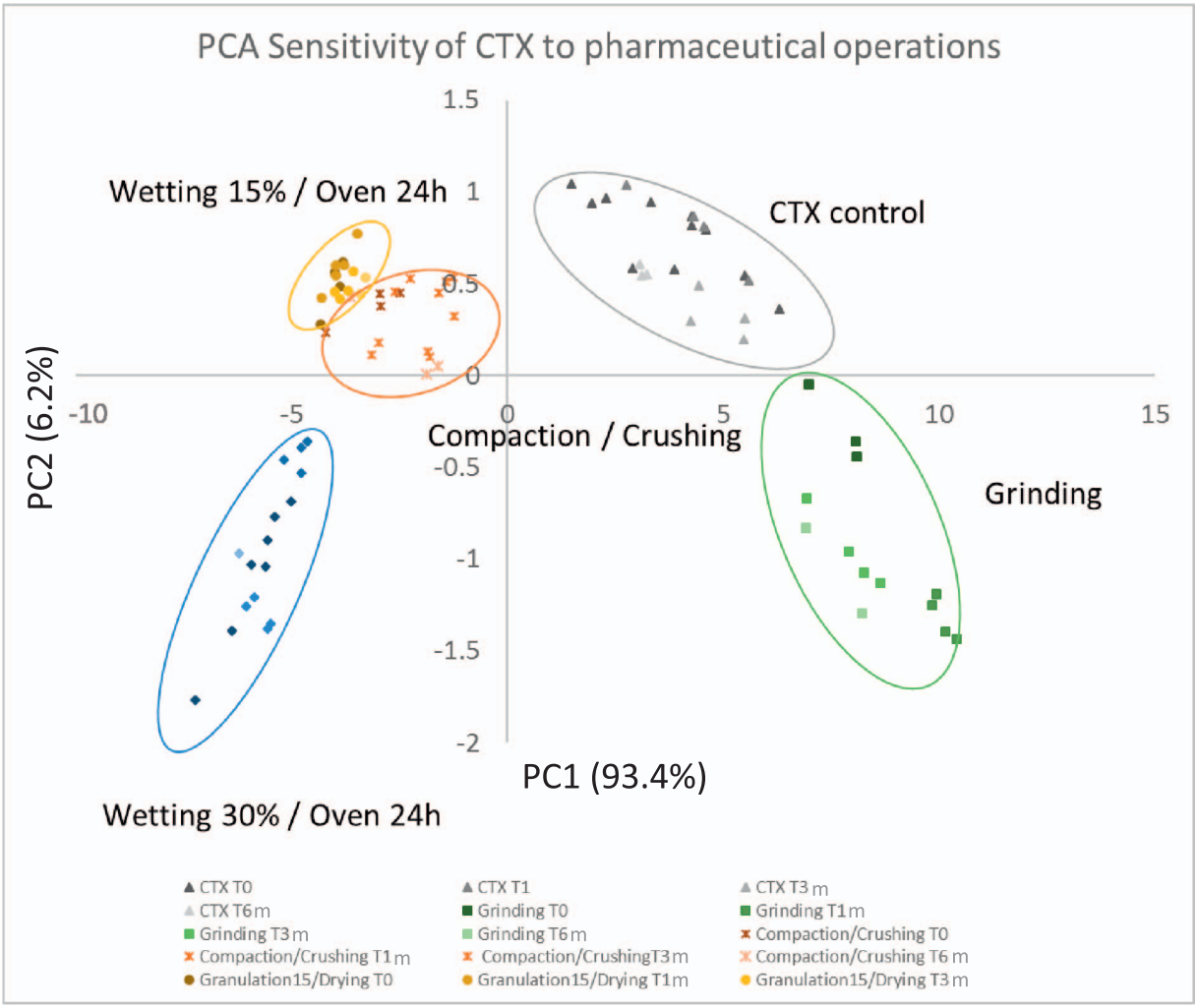

Fig. 5. Scatter plot of PCA model for the pharmaceutical process impact on CTX formulation. Percentage of variance explained is given in parenthesis for the first and the second principal component (93.4% and 6.2% for PC1 and PC2 respectively).

tograms c and d) was probably due to preferential orientation effects, as that observed during axial compression of a crystalline powder. The peak broadening could be due to crystallite size reduction and/or increasing of the micro-strain inside the crystallites, the former may be a signature of crystallites damaging as it corresponds to an increase of the default rate.

Maximum broadening effects were observed for CTX after grinding and wetting with 20% of water (Fig. 7b). Additionally, an increase of the background line was observed for CTX after grinding and after compression with crushing, which could be due to (i) the partial amorphisation of the compound or (ii) the appearance of amorphous by-products due to degradation of CTX (Fig. S4).

A moderate broadening effect was detected in CTX after compression. It is interesting to note that this effect increased with the compression rate (Fig. 7c) reaching a value close to what was observed after wetting.

In order to extract the microstructural effects responsible for this peak broadening, high resolution PXRD diagrams were recorded and refined according to the cell parameters found for the reference compound. The results obtained for CTX after wetting with 20% of water, wetting with 30% of water, dried and CTX tableted are shown in Table 1. The results of the refinement are available in Figs. S1 to S3 in the supplementary information.

As shown clearly in Table 1, the peak broadening was not due to the same effect under different processes, but depended on conditions. While broadening was essentially caused by an increase of the micro-strain for CTX after wetting with 20% of water, it was mostly caused by a decrease of the crystallite size in all other cases. The physical consequences were very different for CTX, as an increase of the micro-strain is associated with an increase of the default rate inside the crystallites that could be due to a damage of the crystalline phase.

To summarise the PXRD analyses of CTX under different conditions, the following conclusions are drawn:

At T0, for all conditions tested, there was no phase transition and the same crystalline phase remained. However, all processes tested affected the microstructural properties of CTX powder, but in varying ways. CTX compression gives rise to smaller crystallite size but does not affect the default rate inside the crystallites, which leads to very limited damage of the crystallites. It was noted that crystallite size decreased when pressure increased.

When CTX was exposed to wetting with 30% of water and dried, the same conclusion was drawn even if a slight increase of the micro-strain was observed; this could reflect the beginning of crystallite degradation which is stopped by the drying process.

Different behaviour is observed when CTX is exposed to wetting with 20% of water without drying. In this case, the default rate increases markedly whereas the crystallite size is less affected. This leads to significant damage of the crystallite, which might indicate that crystallite damage continues when wet CTX is not dried.

The behaviour of CTX under grinding or compression and crushing

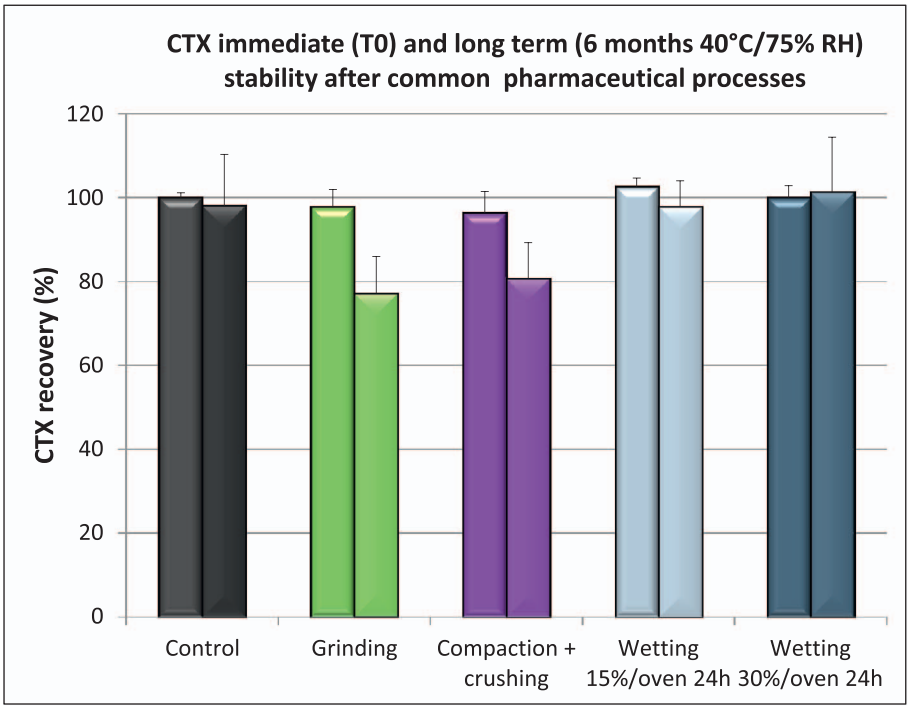

Fig. 6. Impact of pharmaceutical processes on immediate CTX content (first column) and CTX content after 6 months of accelerated aging study (40 °C/75% RH), (second column). ***p*-Value < 0.01 compared to the control; **p*-value < 0.05 compared to the control.

was probably different because amorphisation occurred, which was not detected under any other conditions. This behaviour is most probably due to grinding/crushing effects rather than compression, as compression alone did not lead to significant amorphisation.

In conclusion, the effect of water on CTX clearly induced rapid damage of crystallites. When CTX was dried after water exposure, this damage was halted. These observations correspond with stability results, since the wet granulation process does not induce any significant CTX content decrease. Crushing or grinding of CTX induces the occurrence of an amorphous phase, which could explain the singular behaviour of CTX under these conditions during stability studies.

3.5. Compatibility of CTX with common pharmaceutical excipient

As CTX is commercialized as a pure powder for extemporaneous reconstitution of an injectable solution, little information has been available on CTX compatibility with common pharmaceutical excipients.

Apart from the previously discussed moisture lability, the main source of reported CTX incompatibility data derive from clinical application. Concomitant calcium is reported to be associated with life-threatening adverse drug reactions (Bradley et al., 2009) and have resulted in contra-indication of CTX administration with calcium containing intravenous solutions. A recent review (Donnelly et al., 2017) confirms data underpinning this precaution.

As drug-excipient interactions in solid dosage forms are known (Narang et al., 2012) to affect drug product stability in terms of physical (organoleptic changes and reduction in the dissolution), or chemical aspects (causing drug degradation), additional studies were performed to further explore the compatibility of CTX with various excipients.

A list of 87 common pharmaceutical excipients of solid, liquid and semi-solid drug delivery forms were reviewed for testing in binary mixtures for immediate and long term (6 months at 40 °C/75% RH) CTX compatibility. For each excipient, specificity of the analytical method was checked by injecting the excipient alone. The method was determined to be specific for all excipients tested. These compatibility studies permit establishment of lists of compatible excipients (48 excipients out of 87 tested), incompatible excipients and second line excipients; the latter might be used with caution should no other compatible excipient be available (supplementary material, Table S1).

Among the absorption enhancers tested, all excipients were compatible except Capmul MCM and Labrasol, whose compatibility with the CTX was found to induce degradation of CTX. Sodium chenodeoxycholate was found to be compatible. Most excipients considered for use in solid dosage forms were compatible. Only four excipients – sorbitol, carboxymethylcellulose, gelatin and chitosan – were incompatible with CTX. Liquid excipients presented greater problems of compatibility. In particular, there was a lack of compatibility between CTX and hydrophilic solvents and several emulsifying agents/surfactants. Among liquid forms, Capryol 90, Miglyol 812N, Captex 355 and Capmul PG12 gave acceptable results.

PEG compatibility results showed that PEGs were not suitable for soluble suppositories. For suppositories which melted, the only excipient which could be used was white wax. These lists (supplementary material, Table S1) are likely to be helpful for further pharmaceutical development, using DOE (design of experiments) approaches. However, compatibility studies cannot replace stability studies of final formulations; use of compatible excipients would only reduce the risk of degradation of CTX. All final formulations require further stability tests.

4. Conclusion

The preformulation studies of CTX addressed CTX humidity and water lability, the impact of pharmaceutical processes and compatibility with common excipients. CTX is not highly hygroscopic and might be processed in all climatic zones, but should be packaged against humidity. Water presence in formulations is critical, as CTX long term stability is affected with > 2.8% w/w of added water.

Granulation processes are possible and can improve the CTX bad flowability, provided that compression forces and drying are optimized for dry and wet granulation respectively. Ideally, grinding should be avoided, as this leads to CTX amorphisation and long term degradation. NIR studies and PCA allowed the establishment of models to classify CTX powder according to conditions of exposure or the processes

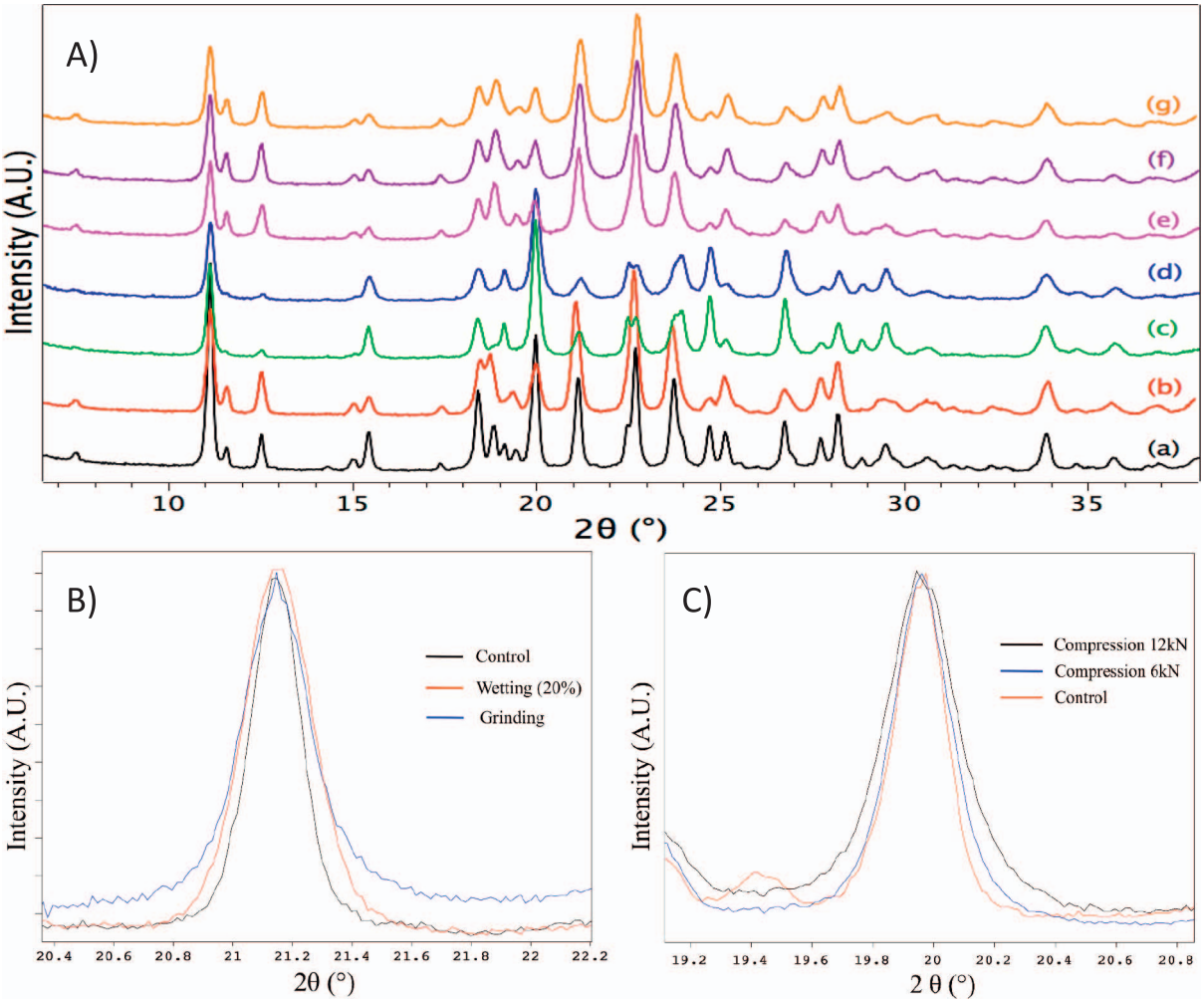

Fig. 7. A) Routine PXRD diagrams of CTX under different conditions: (a) control, (b) wetting with 20% of water, (c) compression 80 MPa, (d) compression 150 MPa, (e) grinding, (f).compression at 80 MPa and crushing, (g) compression at 150 MPa and crushing. B) Zoom on PXRD diagrams of CTX after wetting with 20% of water and after grinding showing the broadening of the peak at $2\theta = 21.14^{\circ}$ compared to the CTX reference diagram. C) Zoom on PXRD diagrams of CTX after compression at 80 MPa (6 kN) and 150 MPa (12 kN) showing the broadening of the peak at $2\theta = 19.94^{\circ}$ compared to the CTX reference diagram.

Table 1
Microstructural parameters extracted from the profile matching refinement of CTX after wetting with 20% of water, wetting with 30% of water and then drying and when CTX is tableted.

Conditions	Mean crystallite size (nm)	Mean crystallite strain ($\times 10^{-4}$)
Control	70	25
CTX wetting 20%	50	50
CTX wetting 30% and drying	55	40
CTX tableted	50	20

Table 2
Main conclusions for future CTX formulation development.

Formulation type	Solid	Liquid
Critical parameters	Flowability + + + Sensitivity to water and humidity + + + Compatibility +	Compatibility + + + Homogeneity of suspensions (anticipated)
Pharmaceutical processes	Wet granulation: GO providing complete drying and reduced time of wetting (fluid bed technology) Dry granulation and compression: GO providing moderate compression forces Grinding: NO GO	Dispersion: GO

+ critical.
+ + + highly critical.

applied. This technique might be valuable for process monitoring. XRD provided deeper insights into degradation processes related to CTX exposure to various conditions. The main conclusions in view of future CTX formulation development, are summarized in Table 2.

The compatibility study shows that for further pharmaceutical development, solid drug dosage forms are more likely to achieve long term stability. Aqueous solutions are unstable and compatible liquid excipients do not dissolve CTX sufficiently. Oily suspensions are an alternative with potentially good stability. Soluble suppositories are not an option because all excipients tested were incompatible, but wax suppositories might be formulated. Chenodeoxycholate as absorption enhancer did not lead to any compatibility issues and could be used in further development studies.

These results suggest that pharmaceutical development of a non-parenteral drug dosage form of CTX is challenging, but feasible. Future drug dosage forms could include tablets, hard capsules, granulates, non-aqueous suspensions, oleo-gels or self-emulsifying drug delivery systems.

Supplementary data to this article can be found online at http://dx.

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